REMARKS

Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-33 and 42-44 are pending. Claims 1, 4, 9, 10, 12, 22-24 and 27 are amended to correct minor language inconsistencies and to clarify the claims to render it clear that the method involves modifying nucleic acid molecules one codon at a time to replace a single amino acid at a time, introducing modified nucleic acid molecules one-by-one into host cells and individually screening each protein that is produced so that each modified protein is expressed and screened separately. This is as described in the application. For example, in the first paragraph of the "Summary," the application states:

> In practicing the methods, each molecule is individually designed, produced, processed, screened and tested in a high throughput format. Neither random or combinatorial methods nor mixtures of molecules are used.

This description is repeated throughout the application; although in the first step, the application describes that the molecules are not necessarily individually designed, but can be prepared by any method. Once designed, molecules, such as nucleic acid molecules are individually expressed, screened and tested.

Claims 1, 22-24 and 27 are amended to recite that the cells are provided **in** an addressable array. This language is employed to advance prosecution. As described in the specification, an addressable array does not necessarily refer to a solid matrix upon which cells or other moieties are localized. As described in the application an array is a collection of elements; and an addressable array is one in which loci thereof can be identified. Such loci and identification is not required to be a positional localization, but can be identifiable by encoding constituents of the collection. See, for example, page 27, line 20, - page 28, line 2:

> As used herein, an array refers to a collection of elements, such as nucleic acid molecules, containing three or more members. An addressable array is one in which the members of the array are identifiable, typically by position on a solid phase support or by virtue of an identifiable or detectable label, such as by color, fluorescence, electronic signal (i.e. radiofrequency (RF), microwave or other frequency that does not substantially alter the interaction of the molecules of interest), bar code or other symbology, chemical or other such label. Hence, in general the members of the array are immobilized to discrete identifiable loci on the surface of a solid phase or directly or indirectly linked to or otherwise associated with the identifiable

label, such as affixed to a microsphere or other particulate support (herein referred to as beads) and suspended in solution or spread out on a surface.

By amendment of the claims herein, such alternative embodiments are not excluded from the scope of the claims. No new matter is added.

THE REJECTION OF CLAIMS 1-29, 32, 33 AND 42-44 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-29, 32, 33 AND 42-44 are rejected under 35 U.S.C. §112, second paragraph, for reasons discussed in turn below. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

Claims 1, 22-24 and 27

Claims 1, 22-24 and are rejected in the recitation of "the host cells are provided as an addressable array" because such language allegedly is unclear. The claims are amended to recite "in an addressable array," in order comply with the suggestion of the Examiner, but such amendment is not intended to limit the scope of the claims nor does Applicant agree that "as an addressable array" is unclear. As discussed above, and described above and in the application, an array is a collection and an addressable array is one in which the members are identifiable. Hence the members of the array can be labeled so that each is identifiable; it is not necessary for the members to physically arrayed on a surface. "In an addressable array" and "as an addressable array" mean the same thing: the members of the collection are identifiable.

Claim 10

Claim 10 is rejected as indefinite in the recitation of "step (d)," rather than the antecedent in claim 9 of "step (e)." This inadvertent error is corrected.

THE REJECTIONS OF CLAIMS 1-33 AND 42-44 UNDER 35 U.S.C. §103

The particular grounds of rejection are discussed in turn below. As discussed below, neither Zlauddin *et al.* nor Short nor any reference of record, nor any combination of references, teaches or suggests step (a) or (d) of the method as claimed I each of the independent claims. In particular, as discussed above, no reference teaches or suggests a method for the identification of a protein that differs in a predetermined property from a target protein that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded

proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). As discussed below, the method of Short necessarily produces a mixture of nucleic acid molecules and also of expressed proteins. In the method of Short mixtures of polypeptides are screened. The polypeptide(s) selected as having a property of interest must be sequenced to identify hit positions. The method does not identify the protein and a hit position in a single step.

Neither Short nor Zlauddin nor any reference provides any suggestion to change the operation of the method of Short, nor any way to modify the saturation mutagenesis method of Short, which relies on degenerate oligonucleotides for modifying each locus in a polypeptide, so that a non-degenerate oligonucleotide is employed. Short specifically teaches that its method provides a way to synthesize all mutations at each locus in a single reaction vessel. The method of Short requires screening of mixtures of proteins and then sequencing to identify a hit position.

Rebuttal to particular points raised by the Examiner appears at the end of the discussion of each ground of rejection.

Claims 1-21, 27, and 42-44

Claims 1-21, 27, and 42-44 are rejected under 35 U.S.C. §102(e) as being unpatentable over Zlauddin *et al.* in view of Short (US Patent No. 6,171,820 B1) because Zlauddin *et al.* allegedly teaches a method that produces a set of nucleic acid molecules that encode the same protein; individually introducing each set of nucleic acid molecules into cells that are displayed on an addressable array so that the cells at each locus contain the same nucleic acid molecule; expressing the encoded protein, and screening the proteins for a particular chemical or biological property. Zlauddin *et al.* allegedly only fails to teach use of its method for molecular evolution, because Zlauddin *et al.* does not teach use of its method for individually producing nucleic acid molecules that differ from a target molecule at a single locus, and screening encoded proteins to identify hits. The Examiner urges that Short supplies the allegedly missing element by teaching a method of site-saturation mutagenesis, which teaches production of modified nucleic acid molecules that each contain at least one substitute codon encoding each of the naturally-occurring amino acids.

The Examiner concludes that it would have been obvious to one of ordinary skill in the art to have combined the methods of Zlauddin *et al.* and Short and have screened the nucleic acid molecules produced in the methods of Short *et al.* using cell microarrays.

This rejection respectfully is traversed. As discussed below, the methods are not amenable to combination so that the combination of teachings of the two references cannot result in the instantly claimed methods. Even if the teachings could be be combined, the combination does not result in the instantly claimed methods. The method of Short *et al.* necessarily results in mixtures of polynucleotides. Zlauddin *et al.* does not teach or suggest any modification of its method or the method of Short in which the mixtures of polynucleotides produced in the method of Short are individually introduced into discrete loci on a lawn of cells, and individually expressed and screened. No method is described in either Short of Zlauddin *et al.* for separating the mixtures of polynucleotides.

As established below, there is no teaching supporting the combination of the references; the combination does not result in the claimed methods because the combination is missing elements, such as how to obtain individual cDNA molecules from the mixtures necessarily produced by the method of Short; there is no suggestion of any desirability for modification of the method of Short; and the proposed modification or combination of the cited references changes the principle of operation of the method of Short (saturation mutagenesis). Consequently for these reasons, discussed in more detail below, and for the additional reasons discussed below, the Examiner has failed to establish a *prima facie* case of obviousness of any pending claim.

RELEVANT LAW

In order to set forth a prima facie case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art

reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Importantly, all claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. See, e.g., MPEP 2143.03 and In re Lowry, 32 F.3d 1579, 32 U.S.P.Q.2d 1031 (Fed. Cir. 1994), citing In re Gulack, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983), citing In re Royka, 490 F.2d 981, 180 U.S.P.Q.2d 580 (CCPA 1974).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

The Claims

Independent Claim 1 and dependent claims are directed to a process for the identification of a protein that differs in a predetermined property from a target protein. The method includes the steps of (a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, **where all members of the set encode the same polypeptide**, (b) **individually** introducing each set of nucleic acid molecules into host cells and expressing the encoded protein, wherein the host cells are present in an addressable array such that all host cells of one loci contain the same nucleic acid molecule express the proteins that have the same modification so that sets of proteins are produced in which the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid; and; and (c) **individually** screening each set of encoded proteins, whereby one or more proteins that have a predetermined property that differs from the target protein is/are identified. The predetermined property is selected among a chemical, a physical and a biological property of the target protein. The identified proteins each are designated as a hit and each hit contains a mutation designated a hit position. Because the proteins are prepared and screened one-by-one, identification of protein having a

predetermined property necessarily identifies the hit position. There is no step of sequencing or otherwise analyzing the identified protein to identify a hit position.

Dependent claims specify variations of the method including methods of designing and/or synthesizing nucleic acids, methods using addressable arrays, solid supports, types of nucleic acid molecules, variations in the nucleic acids, target proteins and predetermined properties used in the methods and addition steps that can be used with the methods. Dependent claims 2-21, 32, 33 and 42-44 recite additional limitations as do independent claims 22-24 and 27.

Claim 27 and dependent claims 28 and 29 similarly recite that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b) and recites additional steps.

Zlauddin *et al.*

Zlauddin *et al.* teaches a method for cell based screening of physical arrays of cDNA molecules. Copies of arrays of cDNA clones are printed onto a lawn of cells under conditions in which the cells are transfected. Zlauddin et al. does not teach or suggest a method in which nucleic acid molecules encoding modified forms of a protein are screened separately nor any modification of the method of Short et al. in which each modified nucleic acid molecule is separately produced, introduced into a cell and tested for a predetermined activity.

Furthermore, contrary to the statement of the Examiner, Zlauddin *et al.* does not teach or suggest how to separate individual cDNA clones from among a mixture of clones. The method of Zlauddin *et al.* starts with individual clones. Individual clones are printed on to a class slide using a robotic arrayer; the slide is then placed in a culture dish covered with adherent mammalian cells in the medium. The cells that grow on the DNA on the slides express the DNA and divide to produce a microarray of transfected cells amongst a lawn of cells.

Zlauddin *et al.* teaches a method for microarraying and expressing individual cDNA clones in a lawn of cells. Zlauddin *et al.* fails to teach or suggest a method for the identification of a protein that differs in a predetermined property from a target protein that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded proteins to

identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)).

Short

Short fails to cure these deficiencies in the method of Zlauddin *et al.* Specifically, Short fails to teach or suggest a method for producing a population of sets of nucleic acid molecules that encode modified forms of a target protein in which all nucleic acid molecules in a set encode the same modified protein (step (a)). Further, Short fails to teach or suggest individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)) and to thereby identify hit positions. Short fails to teach or suggest a method in which the step of identifying a protein also identifies the hit position.

Short is directed to mutagenesis techniques for directed evolution of proteins. Short teaches a method for producing a set of mutagenized progeny polynucleotides encoding a polypeptide from a parental template polynucleotide via codon site-saturation mutagenesis, where at each original codon there is produced at least one substitute codon encoding each of the 20 naturally occurring amino acids. Short describes a saturation mutagenesis method that includes generating a set of modified polypeptides in which a full range of amino acid substitutions is represented at each amino acid position. In the method as taught by Short, **degenerate oligonucleotide cassettes** are used to generate sets of modified polynucleotides encoding the mixtures of modified polypeptides. The patent states (column 34, lines 43-49) that the polynucleotides encoding the modified polypeptides are in a single reaction vessel that contains at least 32 distinct polynucleotides encoding 20 distinct polypeptides.

In practicing the saturation mutagenesis process, degenerate sets of oligonucleotides are prepared (see column 33, line 51, - column 35, line 27) using "proprietary" sets "of progeny polypeptide in which the full range of single amino acid substitutions is represented at each amino acid position." The oligos that are used contain a first homologous sequence, and then a degenerate N, N, G/T sequence (defined at col. 12, lines 25-27, to be a "nucleotide sequence representing all possible triplets, where N is A,C, G or T"), and optionally a second homologous sequence. The progeny translational products include all possible amino acid changes at each amino acid site along the polypeptide. The products are produced by using the degenerate oligos. The patent states (col. 33, line 66, - col. 34, line 15):

In one aspect, one such degenerate oligo (comprised of one degenerate N, N, G/T cassette) is used for subjecting each codon in a parental polynucleotide template to a full range of codon substitutions. In another aspect, at lest two degenerate cassettes are used –either in the same oligo or not, for subjecting at least two original codons in a parental template to a full range of codon substitutions.. . .

. . . Thus, ***in a reaction vessel*** in which a parental polynucleotide sequence is subjected to saturation mutagenesis using one such oligo, there are ***generated 32 distinct progeny*** polynucleotides encoding 20 distinct polypeptides. **In contrast, the use of a non-degenerate oligo in site-directed mutagenesis leads to *only one progeny polypeptide product per reaction vessel* .**[emphasis added]

This mixture of polynucleotides is transformed into host cells, which express the encoded polypeptides, and the cells are screened. As result **each set** (each reaction vessel) contains a mixture of different modified polypeptides. The method necessarily yields mixtures of progeny polynucleotides and ultimately mixtures of polypeptides. The purpose of the method of the application to generate mixtures. The host cells express mixtures of polypeptides, which mixtures are screened. Once screened an a polypeptide of interest identified, it must be sequenced to identify a hit position. Hence the polypeptides are not individually expressed nor are they individually screened, nor does Short provide a method in which modified polypeptides are individually produced nor one in which identification of the protein with a predetermined property also identifies the hit position.. .

Hence, the method the saturation mutagenesis method of Short **necessarily** results in the preparation of mixtures modified nucleic acid molecules and ultimately in mixtures of polypeptides. The methods employ a degenerate oligonucleotide (at least 32-fold) for production of the modified nucleic acid molecules and then the progeny polypeptides. There is no method taught or suggested in Short that would result in single mutagenized nucleic acid molecules that could be individually introduced into host cells to produce polypeptides that could be individually screened; the methods necessarily result in mixtures of nucleic acid molecules and mixtures of polypeptides. The method of Short is **not amenable** to individually introducing sets of nucleic acid molecules into host cells to produce sets containing the same polypeptide and screening each set separately. Short teaches no method to so; and in fact touts the advantages of its method to prior methods of site mutagenesis as providing generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides in a single reaction vessel.

There is no suggestion of any desirability for modification of the method of Short in Short or in Zlauddin *et al.* The proposed modification or combination of the prior art as set forth by the Examiner would change the principle of operation of the method of Short, which is designed to produce mixtures of polynucleotides and then mixture of polypeptides. Furthermore, the combination of teachings of Zlauddin *et al.* and Short does not result in the instantly claimed methods, since elements (a) and (d) are missing. Hence the Examiner has failed to set forth a *prima facie* case of obviousness.

ANALYSIS

1. There is no suggestion of any desirability for modification of the method of Short in Short or in Zlauddin *et al.* The proposed modification or combination of the prior art as set forth by the Examiner would change the principle of operation of the method of Short, which relies upon the use of degenerate oligonucleotides in a single reaction vessel to produce the mixtures of modified nucleic acid molecules and expressed proteins. If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). Since the proposed modification and/or combination of teachings of Zlauddin *et al.* and Short would change the basic operation of the method of Short (to produce mixtures of polynucleotides), the claimed methods cannot be *prima facie* obvious.

2. The combination of teachings of Zlauddin and Short does not resulting the instantly claimed methods

The combination of teachings of Zlauddin *et al.* and Short does not result in the instantly claimed methods, since elements (a) and (d) are missing. Hence the Examiner has failed to set forth a *prima facie* case of obviousness.

As discussed above, Zlauddin *et al.* fails to teach or suggest a method that is for the identification of a protein that differs in a predetermined property from a target protein and fails to teach or suggest such a method that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)) and thereby identifying a hit position. The method of Zlauddin *et al.* starts with individual

cDNA clones; Zlauddin et al. does not teach or suggest a method of mutagenesis that generates the individual clones nor a method for separating the mixtures of nucleic acid molecules produced by the saturation mutagenesis method.

Short fails to provide the missing elements. In particular, Short does not provide a method that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, **where all nucleic acid molecules in a set encode the same modified protein** (step (a)). As described above, the method of Short necessarily and inevitably produces a mixture of nucleic acid molecules that are introduced into cells to produce a mixture of expressed proteins in the same reaction vessel. The method relies on saturation mutagenesis which uses degenerate oligonucleotides are primers. Short specifically and unequivocally states that its methods is designed to produce mixtures of mutated nucleic acid molecules and polypeptides (col. 34, lines 30-49):

> It is appreciated, however, that the use of a degenerate N,N,G/T triplet as disclosed in the instant invention is advantageous for several reasons. In one aspect, this invention provides a means to systematically and fairly easily generate the substitution of the full range of possible amino acids (for a total of 20 amino acids) into each and every amino acid position in a polypeptide. Thus, for a 100 amino acid polypeptide, the instant invention provides a way to systematically and fairly easily generate 2000 distinct species (i.e. 20 possible amino acids per position X 100 amino acid positions). It is appreciated that there is provided, through the use of an oligo containing a degenerate N, N,G/T triplet, 32 individual sequences that code for 20 possible amino acids. T***hus, in a reaction vessel in which a parental polynucleotide sequence is subjected to saturation mutagenesis using one such oligo, there are generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides***. In contrast, the use of a non-degenerate oligo in site- directed mutagenesis leads to only one progeny polypeptide product per reaction vessel. [emphasis added]

Hence the method of Short **is not amenable to modification** such that each nucleic acid molecule is separately produced, introduced into host cells and screened. Further, Short **does not suggest the desirability** of such modification, since it touts the advantages of its methods that employ a degenerate oligonucleotide to produce mixtures. The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 USPQ2d 1780 (fed. cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 USPQ43 (CCPA 1963). Short is directed to a method of saturation mutagenesis to produce **mixtures** not

individual modified oligonucleotides nor individually modified nucleic acid molecules encoding modified polypeptides nor sets of single polypeptides.

Short also fails to teach a method of individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). Mixture of proteins are screened. The method of Short results in *mixtures* of cells. Zlauddin *et al.* fails to teach or suggest screening for proteins that differ in a predetermined property, and Short teaches a method that necessarily results in a mixture of proteins.

There is no way to combine methods of Zlauddin *et al.* with those of Short that result in the instantly claimed methods. Therefore, the combination of teachings of references fails to result in the instantly claimed methods. All claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. See, e.g., MPEP 2143.03 and In re Lowry, 32 F.3d 1579, 32 U.S.P.Q.2d 1031 (Fed. Cir. 1994), citing In re Gulack, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983), citing In re Royka, 490 F.2d 981, 180 U.S.P.Q.2d 580 (CCPA 1974). Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

The Combination of References is based on the improper use of Hindsight

"To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 U.S.P.Q. 303, 312-13 (Fed. Cir. 1983). In order to have combined the teachings of Zlauddin *et al.* with those of Short to have arrived at the instantly claimed methods, the Examiner must have read the references in light of the instant application, which teaches preparing the nucleotides individually, producing the encoded polypeptides individually, one and then screening them individually to identify polypeptides (proteins) with predetermined properties and to thereby identify hit positions.

The teachings of the combination of references do not result in the instantly claimed methods. For the combination of teachings to result in the methods as claimed, requires use of the teachings of the application at issue. To produce the claimed methods, requires picking and choosing portions of methods taught in the cited references, combining them as claimed in the application and adding teachings from the instant application.

For example, it is inappropriate for the Examiner to pick the part of Zlauddin *et al.* directed to individually screening clones and part of Short, directed to methods of directed evolution of proteins, and add the missing element, complete modification of the method of Short that is neither taught nor suggested in the cited references to produce the methods as instantly claimed. It is only by reading the instant application, that one of ordinary skill in the art could possibly have combined Zlauddin *et al.* and Short and result in the instantly claimed methods. Short specifically teaches methods that result in mixtures of polypeptides; one of ordinary skill in the art would not in view of Zlauddin *et al.* have modified the method of Short and to produce sets of nucleic acid molecules that encode modified forms of a target protein, where all nucleic acid molecules in a set encode the same modified protein nor any method in which the expressed proteins are screened one-by-one. Further, neither Zlauddin *et al.* nor Short suggests any way to do so.

Claims 22 and 23

Claims 22 and 23 are rejected under 35 U.S.C. §103(a) as being unpatentable over the combination of teachings of Zlauddin *et al.* in view of Short and further in view of Collett *et al.* (US2002/0081574B1) because Collett *et al.* teaches screening modified proteins that exhibit a 10-25 fold change in activity so that it would have been obvious to one of ordinary skill in the art to have combined the methods of Short *et al.* with those of Collett *et al.* since one of ordinary skill in the art would have been motivated to determine the activity of a modified protein. This rejection respectfully is traversed.

Claims

Independent claim 22 recites essentially the same steps as claim 1 and further recites that a "change in a predetermined property comprises a change in an activity of the target protein that is at least about 10%, 20%, 30%, 40% or 50% compared to the unmodified target protein." Independent Claim 23 recites that the change is "at least about 75%, 100%, 200%, 500% or 1000% compared to the unmodified target protein."

Analysis

As discussed above, the combination of teachings of Zlauddin *et al.* with those of Short fails to teach or suggest teach all limitations of claim 1 and hence fails to teach all elements of claim 22. Collett *et al.* fails to cure the deficiencies of Zlauddin *et al.* in view of Short, since Collett *et al.* does not teach or suggest a method for the identification of a protein that differs in a predetermined property from a target protein that includes a step of producing

a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). Therefore the combination of teachings of Zlauddin *et al.*, Short and Collett *et al.* does not result in the methods of claims 22 and 23.

Claims 24 and 27-29

Claims 24 and 27-29 are rejected under 35 U.S.C. §103(a) as being unpatentable over the combination of teachings of Zlauddin *et al.* in view of Short and further in view of Berlioz *et al.* (U.S. Patent No. 5,925,565) because Berlioz *et al.* teaches targeting proteins involved in viral replication so that it would have been obvious to one of ordinary skill in the art to have combined the methods of Short *et al.* with those of Berlioz *et al.*, which teaches that it is a goal to "create the efficient and stable expression of genes. One of ordinary skill in the art would have been motivated to "test the efficiency and stability of vectors" This rejection respectfully is traversed.

Claims

Independent claim 24 includes the method of claim 1 and recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b).

Claim 27 similarly recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b) and recites additional steps.

Analysis

As discussed above, the combination of teachings of Zlauddin *et al.* with those of Short fails to teach or suggest teach all limitations of claim 1 and hence fails to teach all elements of claim teach all elements of claim 24 or claim 27. Berlioz *et al.* fails to cure the deficiencies of Short, since Berlioz *et al.* does not teach or suggest a method that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). Therefore the combination of teachings of Zlauddin *et al.*, Short and Berlioz *et al.* does not result in the methods of claims 22 and 23.

Claims 32 and 33

Claims 32 and 33 are rejected under 35 U.S.C. §103(a) as being unpatentable over the Zlauddin *et al.* in view of Short because use of a computer to automate a process does not without more render a claim unobvious. This rejection respectfully is traversed.

As discussed above, the combination of teachings of Zlauddin *et al.* and Short do not result in a method that includes a step of producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein (step (a)) ; and individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). Therefore, the methods of claims 32 and 33 are not taught or suggested by the combination of teachings of Zlauddin *et al.* and Short.

Rebuttal to specific points noted by the Examiner

In addition to the points discussed above, the Examiner provided arguments directed to dependent claims. These are addressed in turn as follows:

1. **Claim 2 and 10**

The Examiner alleges that Short teaches that each nucleic acid molecule is individually designed and synthesized and points to the Abstract; col. 1, lines 32-42; col. 5, lines 12-33; and columns 33-35. As demonstrated below, none of the cited passages teaches or suggests individually designing and synthesizing each nucleic acid molecule. As discussed above, the saturation mutagenesis method of Short is designed to simultaneously synthesize at least all of the mutations at one locus at a time, so that each reaction vessel contains at a minimum a mixture of 20 different molecules.

a. Abstract - the Abstract states:

> Disclosed is a rapid and facilitated method of producing from a parental template polynucleotide, a set of mutagenized progeny polynucleotides whereby at each original codon position ***there is produced at least one substitute codon encoding each of the 20 naturally encoded amino acids***. Accordingly, there is also provided a method of producing from a parental template polypeptide, ***a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position***. The method provided is termed site-saturation mutagenesis, or simply saturation mutagenesis, and can be used in combination with . . .. [emphasis added]

Hence, contrary to the statement of the Examiner, the abstract states that the method of saturation mutagenesis produces a "a set of mutagenized progeny polynucleotides whereby ***at***

each original codon position there is produced at least one substitute codon encoding each of the 20 naturally encoded amino acids" from which are produced from a parental template polypeptide, a **set** of mutagenized progeny **polypeptides** wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position. The method produces a mixture of at least 20 different mutagenized nucleic acid molecules and ultimately a mixture of at least 20 different polypeptides. The abstract does not disclose production of a set of nucleic acid molecule where all nucleic acid molecules in a set encode the same modified protein

b. Col. 1, lines 32-42, recite:

> This invention relates to the field of protein engineering. More specifically, this relates to a directed evolution method for preparing a polynucleotides encoding polypeptide, which method comprises the step of generating site-directed mutagenesis optionally in combination with the step of polynucleotide chimerization, the step of selecting for potentially desirable progeny molecules (which may then be screened further), and the step of screening the polynucleotides for the production of polypeptide(s) having a useful property.

This paragraph does not teach or suggest individually designing and synthesizing each set of nucleic acid molecules, where all nucleic acid molecules in a set encode the same modified protein.

c. Col 5, lines 12-33, recite:

> In a preferred embodiment, there is generated (e.g. from a parent polynucleotide template)--in what is termed "codon site-saturation mutagenesis" --***a progeny generation of polynucleotides, each having at least one set of up to three contiguous point mutations (i.e. different bases comprising a new codon), such that every codon (or every family of degenerate codons encoding the same amino acid) is represented at each codon position.*** Corresponding to-- and encoded by--this progeny generation of polynucleotides, ***there is also generated a set of progeny polypeptides, each having at least one single amino acid point mutation.*** In a preferred aspect, there is generated--in what is termed "amino acid site-saturation mutagenesis"--one such mutant polypeptide for each of the 19 naturally encoded polypeptide-forming alpha-amino acid substitutions at each and every amino acid position along the polypeptide. This yields--***for each and every amino acid position along the parental polypeptide-- a total of 20 distinct progeny polypeptides*** including the original amino acid, or potentially more than 21 distinct progeny polypeptides if additional amino acids are used either instead of or in addition to the 20 naturally encoded amino acids. [emphasis added]

This paragraph does not teach or suggest individually designing and synthesizing each set of nucleic acid molecules, where all nucleic acid molecules in a set encode the same modified protein. Rather it states that a **set of a mixture of nucleic acid molecules** in which each codon (or every family of degenerate codons encoding the same amino acid) is represented at each codon position is produced and that a set of polypeptides containing at least 20 members in the set is produced.

d. Cols. 33-35, not reproduced in their entirety, describe the process of saturation mutagenesis discussed above and throughout this response. Degenerate oligonucleotides primers (containing a degenerate N, N, G/T sequence) are used to introduce all possible amino acids at each locus. In the simplest embodiment, this is done one locus at a time. Even where one locus is mutated, the method produces a mixture of at least twenty different polypeptides and then twenty encoded polypeptides.

Nowhere in this section does the patent teach or suggest using a single oligonucleotide primer; the entire purpose of the method of saturation mutagenesis is to produce a plurality of mutations at once. In fact, as discussed above, the method is described in contrast to site-directed mutagenesis.

2. Claims 3, 5, 6 and 8

The Examiner urges that Zlauddin *et al.* teaches that each set is deposited at a locus in an array. It is correct that Zlauddin *et al.* teaches depositing nucleic acid molecules in a lawn of cells, in a reverse transfection procedure. The starting point for the Zlauddin *et al.* method is individual cDNA clones. Zlauddin *et al.* does not teach or suggest how such are obtained, and certainly does not suggest a modification of the saturation mutagenesis method to produce individual clones instead of the mixtures that the method is ***designed and intended*** to produce.

3. Claim 4 and 7

The Examiner states that Short teaches that each polynucleotide in a set differs in at least one amino acid from the parent target protein. Whether or not Short states that, Short (see discussion of the cited passages above) also teaches that each set contains, not the same polynucleotide as required by the instant claims, but a mixture of at least 20 different polynucleotides, not the same polynucleotides as required by the instant claims, and that the mixture of polynucleotides that encode proteins that differ in one amino acid from the target and from each other. In the instantly claim methods, the proteins that are produced all

include the same mutation.

4. Claims 11-15, 19 and 42-44

The Examiner states that Short teaches producing nucleic acid molecules by site-directed mutagenesis, citing the Abstract; col. 1, lines 32-42; col. 5, lines 12-33; and columns 33-35, discussed above.

Applicant respectfully disagrees. Short teaches producing modified nucleic acid molecules using saturation mutagenesis in which degenerate primers are used to produce mixtures of modified nucleic acid molecules that encode and ultimately result in mixtures of polypeptides. Short does not teach or suggest producing "a population of sets of nucleic acid molecules that encode modified forms of a target protein, wherein all nucleic acid molecules in a set encode the same modified protein" as required by the instant claims. No where does Short suggest performing a method in which nucleic acids molecules are prepared individually, and expressed individually and screened individually.

5. Claims 16-18

The Examiner states that Short teaches producing nucleic acid molecules by replacing each codon in a position with another codon encoding another amino acid; recombining the nucleic acid molecules; introducing them into cells and screening the cells, and then (citing col. 11, lines 38-46; col. 20, lines 51-58; cols. 55-56, starting a line 34; and col. 5 lines 8-11) recombining two, three or more amino acids.

First, as discussed above, Short teaches producing sets of nucleic acid molecules in which each codon in a position with another codon encoding another amino acid using saturation mutagenesis in which all such changes are effected using degenerate oligonucleotides in a single reaction vessel. As discussed, saturation mutagenesis is described as a method in which "proprietary codon primers (containing a degenerate N, N, G/T sequence)" are used to generate a **set** of progeny polypeptides in which a **"full range** of amino acid substitutions is represented at each amino position."

Regarding further recombinations of hits, the instant claims recite producing polypeptides that contain two or more mutations in which each corresponds to a mutation identified in the basic method as a hit.

a. Col. 11, lines 38-46, is directed to the definition of "isolated nucleic acid," and recites (quoting col. 11, lines 33-49):

> By "isolated nucleic acid" is meant a nucleic acid, e.g., a DNA or RNA molecule, that is not immediately contiguous with the 5' and 3' flanking sequences with which it normally is immediately contiguous when present in the naturally occurring genome of the organism from which it is derived. The term thus describes, for example, a nucleic acid that is incorporated into a vector, such as a plasmid or viral vector; a nucleic acid that is incorporated into the genome of a heterologous cell (or the genome of a homologous cell, but at a site different from that at which it naturally occurs); and a nucleic acid that exists as a separate molecule, e.g., a DNA fragment produced by PCR amplification or restriction enzyme digestion, or an RNA molecule produced by in vitro transcription. The term also describes a recombinant nucleic acid that forms part of a hybrid gene encoding additional polypeptide sequences that can be used, for example, in the production of a fusion protein.

Hence, the cited passage describes what is meant by an isolated nucleic acid molecule and indicates that the term also encompasses nucleic acid molecules encoding fusion proteins. In the instantly claimed methods, a superlead is not a fusion protein per se, but is a protein that includes at least two mutations that have been identified as leads.

b. Col. 20, lines 51-58

> As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; and plant cells. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein

This cited passage seems to be irrelevant to the Examiner's argument.

c. Cols. 55-56, starting a line 34

This citation refers to Example 5, which exemplifies application of saturation mutagenesis to every residue of a 316 amino acid long dehalogenase enzyme and employs 32-fold degenerate primers. In the example, primers were prepared to randomize each codon and were then added to the reaction mixture to produce a mixture of polynucleotides. An aliquot of the mixture of polynucleotides were used to transfer cells, which then were plated and screened to identify polypeptides exhibiting increased thermal stability. Nine single site mutations were identified. **Sequence analysis was performed to identify the amino acids at each locus responsible for improvement.** After sequencing seven loci were identified. Mutants containing a plurality of mutations were prepared.

As with the sections discussed above, this Example shows that the saturation mutagenesis procedure is performed using degenerate oligonucleotides to produce sets of

polynucleotides that encode differerent polypeptides. The instant claims require producing sets of nucleic acids, where "all nucleic acid molecules in a set encode the same modified protein." Furthermore, identification of a protein with the predetermined property necessarily identifies the hit position. The instantly claimed methods do not include a step of sequencing an identified polypeptide to thereby identify a hit position.

d. Col. 5, lines 8-11, recite:

> 3) optionally obtaining &/or cataloguing structural &/or and functional information regarding the parental &/or progeny generation molecules; and 4) optionally repeating any of steps 1) to 3).

This recitation does not teach or suggest the missing elements. Thus, as discussed throughout, Short does not suggest producing sets of nucleic acid molecules that encode modified forms of a target protein, where all nucleic acid molecules in a set encode the same modified protein. The methods of Short necessarily result in sets of nucleic acid molecules that encoded modified forms of a target protein in which the nucleic acid molecules in a set encode **different** modified proteins. The whole purpose of the method of Short and the principle upon which it is based (saturation mutagenesis) is to produce such mixtures. Nothing in Zlauddin *et al.* nor in any reference of record suggests changing the principle upon which the method of Short is based.

If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). Therefore for these reasons and those discussed above, the Examiner has failed to set forth a *prima facie* case of obviousness for any of the pending claims.

* * *

In view of the above, reconsideration and allowance are respectfully requested

Respectfully submitted,

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